

WHAT IS CLAIMED IS:

1. A method of making a tobacco product that has a reduced potential to contribute to a tobacco-related disease comprising:

(a) providing a first tobacco that comprises a compound that contributes to a tobacco-related disease;

(b) obtaining smoke or a smoke condensate from said first tobacco;

(c) contacting a first isolated population of cells with said smoke or smoke condensate from said first tobacco;

(d) identifying a first gene that is expressed in said first population of cells in response to said contact with said smoke or smoke condensate from said first tobacco, wherein expression of said first gene contributes to a tobacco-related disease;

(e) providing a second tobacco that has been modified to reduce expression of a second gene;

(f) obtaining smoke or a smoke condensate from said second tobacco;

(g) contacting a second isolated population of cells with said smoke or smoke condensate from said second tobacco;

(h) identifying a reduction in expression of said first gene that contributes to a tobacco-related disease in said second population of cells, which are contacted with said smoke or smoke condensate from said second tobacco; and

(i) making said tobacco product from said second tobacco, wherein said tobacco product comprising said second tobacco has a reduced potential to contribute to a tobacco-related disease as compared to a tobacco product comprising said first tobacco.

2. The method of Claim 1, wherein said first tobacco is a burley tobacco.

3. The method of Claim 1, wherein said first tobacco is a flue tobacco.

4. The method of Claim 1, wherein said first tobacco is an oriental tobacco.

5. The method of Claim 1, wherein said first and second populations of cells are contacted with smoke.

6. The method of Claim 1, wherein said first and second populations of cells are the same cell type.

7. The method of Claim 1, wherein said first and second populations of cells are immortal cells.

8. The method of Claim 1, wherein said first and second populations of cells are normal human cells of the lung, mouth, or tongue.

9. The method of Claim 1, wherein said first and second populations of cells are normal human bronchial epithelial (NHBE) cells.

10. The method of Claim 1, wherein said second gene that has been modified in said second tobacco is a gene in a pathway of nicotine synthesis.

11. The method of Claim 10, wherein said second gene is selected from the group consisting of putrescine N-methyltransferase, N-methylputrescine oxidase, ornithine decarboxylase, S-adenosylmethionine synthetase, NADH dehydrogenase, phosphoribosylanthranilate isomerase, and quinolate phosphoribosyl transferase (QPTase).

12. The method of Claim 10, wherein said second gene is quinolate phosphoribosyl transferase (QPTase).

13. The method of Claim 10, wherein said second gene is putrescine methyltransferase (PMTase).

14. The method of Claim 1, wherein said modification of said second gene in said second tobacco is a genetic modification.

15. The method of Claim 1, wherein said modification of said second tobacco is a chemical treatment.

16. The method of Claim 1, wherein said tobacco-related disease is selected from the group consisting of pulmonary disease, cardiovascular disease, and cancer.

17. The method of Claim 1, wherein said tobacco related disease is cancer.

18. The method of Claim 1, wherein said first gene has a sequence selected from the group consisting of NM_004261, NM_000859, AK025736, NM_002526, NM_001109, NM_005891, NM_006409, NM_018445, NM_001284, NM_000485, NM_007002, NM_006829, NM_001667, NM_000693, NM_001635, NM_001657, NM_001145, NM_000700, NM_005139, NM_001154, NM_004034, NM_016476, NM_016085, NM_005721, NM_017900, M90355, NM_004281, NM_001196, NM_003860, NM_014567, NM_021096, NM_005186, NM_001750, NM_013376, NM_015965, NM_016041, NM_016038, BC002971, NM_006429, NM_000647, NM_012111, AK026450, NM_007096, BC010039, NM_016451, NM_007263,

NM_004645, AL162070, NM_000389, NM_000099, NM_001554, NM_007274, NM_020189, NM_004396, NM_001357, AB040961, NM_007326, NM_020548, NM_013253, NM_004405, AL080156, NM_014045, NM_001539, NM_006145, NM_004419, NM_001946, NM_014390, NM_005451, NM_004092, NM_004431, NM_016357, BF541376, NM_003757, NM_003755, NM_001417, NM_004095, NM_005243, NM_005245, NM_004104, AK054816, NM_001457, NM_014164, AL365404, NM_007278, NM_001520, AK024486, NM_001498, NM_002061, NM_004446, NM_002064, NM_002083, NM_000637, NM_002087, L24498, NM_006644, NM_002157, NM_005345, NM_006597, NM_004134, NM_016292, NM_002133, NM_004712, NM_001533, AK057120, AF130111, NM_001536, AK023395, AK054711, AK055071, AK056736, AK024927, AK055564, AK026181, AK026902, AL512727, AL117595, AL050378, AF041429, AF118072, AF065241, BC010009, BC011880, BC017001, BC007307, NM_014029, NM_014047, AF161415, NM_016099, NM_014168, NM_014182, AL139112, AL354915, NM_000182, NM_016404, NM_016623, NM_015932, NM_015343, AF103803, NM_014886, NM_018437, NM_018306, NM_032813, NM_022842, NM_031207, NM_024508, AK027859, NM_032771, BC014850, NM_032899, NM_024040, NM_024038, NM_031943, NM_052815, NM_016545, NM_005542, NM_021999, NM_006147, NM_000576, Z17227, NM_004508, NM_005354, NM_006854, NM_000421, NM_000224, NM_005555, NM_014815, NM_000899, NM_001730, NM_003937, NM_005558, NM_016201, NM_015925, NM_014463, NM_004995, NM_005916, NM_006428, NM_006636, NM_004528, NM_022818, NM_014341, NM_014161, NM_021134, NM_017446, NM_021210, NM_004529, NM_033546, AB032945, NM_017534, NM_002473, NM_002356, NM_000903, NM_004541, NM_004548, NM_004547, NM_002494, NM_014328, BC010285, NM_000271, NM_006096, NM_006164, NM_003489, NM_017838, NM_002820, NM_020992, NM_002574, NM_003713, NM_002631, NM_002632, NM_002658, NM_014287, NM_003819, NM_000937, NM_001198, NM_002583, NM_000917, NM_053024, AB051437, NM_002778, NM_000963, BC013908, NM_002806, NM_002815, NM_002812, NM_002797, NM_002799, NM_014330, NM_004156, NM_006808, NM_015714, BC012513, NM_003979, NM_001666, NM_001033, NM_002950, NM_001029, NM_002953, AB037819, NM_014248, NM_006743, NM_004902, NM_000687, AB051532, NM_003900, NM_001085, NM_030666, NM_000602, NM_015966,

NM_006622, AB000462, NM_003134, NM_003145, NM_007107, AF395440, NM_005870, NM_006109, NM_015523, NM_030981, NM_006518, NM_005628, NM_004207, NM_018976, NM_014331, NM_003130, NM_004599, NM_006745, NM_006918, NM_006819, NM_006704, NM_002999, NM_006289, NM_015641, NM_003217, NM_003314, NM_003329, NM_003330, NM_004238, NM_006755, NM_003234, NM_001064, NM_012459, NM_006470, NM_003449, NM_003289, NM_003404, NM_012321, M26880, NM_014501, NM_003334, AL110132, BC007657, NM_003364, NM_003574, NM_012323, NM_002359, NM_002467, NM_006007, NM_013360, and NM_004234.

19. The method of Claim 1, wherein said first gene has a sequence selected from the group consisting of: NM_00359, NM_00405, NM_00521, NM_00626, NM_01225, NM_00482, NM_00284, AF308602, NM_01438, NM_00371, NM_00164, NM_01633, NM_01865, NM_01242, AF156165, NM_00205, AF163473, NM_03328, AK024486, NM_00343, U18018, NM_00523, BC013971, AJ420488, NM_00548, NM_00578, NM_00094, NM_00675, NM_00228, AL110274, NM_01428, NM_01787, and NM_00437.

20. The method of Claim 1, wherein said first gene is selected from the group consisting of Cullin 4A, C-jun, Hoxa10, and PPP2R1B.

21. The method of Claim 1, wherein said tobacco product is a cigarette.

22. A tobacco product made by the method of Claim 1.

23. A method of reducing the potential of a tobacco consumer to acquire a tobacco-related disease comprising providing the tobacco product made by the method of Claim 1 to said tobacco consumer.

24. Use of the method of Claim 1 to prepare a tobacco product that has a reduced potential to contribute to a tobacco-related disease.

25. A method of making a tobacco product that has a reduced potential to contribute to a tobacco-related disease comprising:

- (a) providing a first tobacco that comprises a compound that contributes to a tobacco-related disease;
- (b) obtaining smoke or a smoke condensate from said first tobacco;
- (c) contacting a first isolated population of cells with said smoke or smoke condensate from said first tobacco;

(d) identifying a first gene that has reduced expression in said first population of cells in response to said contact with said smoke or smoke condensate from said first tobacco, wherein said reduced expression of said first gene contributes to a tobacco-related disease;

(e) providing a second tobacco that is the same variety and grown under the same conditions as said first tobacco, wherein said second tobacco has been modified to reduce expression of a second gene;

(f) obtaining smoke or a smoke condensate from said second tobacco;

(g) contacting a second isolated population of cells with said smoke or smoke condensate from said second tobacco;

(h) identifying an up-regulation in expression of said first gene in said second population of cells, which are contacted with said smoke or smoke condensate from said second tobacco; and

(i) making said tobacco product from said second tobacco, wherein said tobacco product comprising said second tobacco has a reduced potential to contribute to a tobacco-related disease as compared to a tobacco product comprising said first tobacco.

26. The method of Claim 25, wherein said first tobacco is a burley tobacco.

27. The method of Claim 25, wherein said first tobacco is a flue tobacco.

28. The method of Claim 25, wherein said first tobacco is an oriental tobacco.

29. The method of Claim 25, wherein said first and second populations of cells are contacted with smoke.

30. The method of Claim 25, wherein said first and second populations of cells are the same cell type.

31. The method of Claim 25, wherein said first and second populations of cells are immortal cells.

32. The method of Claim 25, wherein said first and second populations of cells are normal human cells of the lung, mouth, or tongue.

33. The method of Claim 25, wherein said first and second populations of cells are normal human bronchial epithelial (NHBE) cells.

34. The method of Claim 25, wherein said second gene that has been modified in said second tobacco is a gene in a pathway of nicotine synthesis.

35. The method of Claim 34, wherein said second gene is selected from the group consisting of putrescine N-methyltransferase, N-methylputrescine oxidase, ornithine decarboxylase, S-adenosylmethionine synthetase, NADH dehydrogenase, phosphoribosylanthranilate isomerase, and quinolate phosphoribosyl transferase (QPTase).

36. The method of Claim 35, wherein said second gene is quinolate phosphoribosyl transferase (QPTase).

37. The method of Claim 35, wherein said second gene is putrescine methyltransferase (PMTase).

38. The method of Claim 25, wherein said modification of said second gene in said second tobacco is a genetic modification.

39. The method of Claim 25, wherein said modification of said second tobacco is a chemical treatment.

40. The method of Claim 25, wherein said tobacco-related disease is selected from the group consisting of pulmonary disease, cardiovascular disease, and cancer.

41. The method of Claim 25, wherein said tobacco related disease is cancer.

42. The method of Claim 25, wherein said first gene has a sequence selected from the group consisting of NM_006856, NM_001143, NM_001657, AB053314, AK023086, BI820294, AK025253, NM_001271, NM_006589, AK000796, NM_001934, NM_005509, NM_004419, NM_003494, NM_000145, NM_005708, NM_002053, AB033063, NM_002129, NM_003542, NM_024598, NM_017933, NM_024037, BC016840, AK027858, NM_006903, NM_000526, NM_000424, NM_005554, NM_005556, AK024583, NM_005583, AL137524, AL117623, NM_012334, AB007959, NM_002520, NM_033014, NM_024594, AB029015, NM_018049, BC015542, NM_018936, NM_000320, NM_000456, NM_007273, NM_005978, NM_016372, NM_006456, NM_024624, AL353933, AK027663, AK024451, NM_005480, NM_002466, NM_006385, NM_005096, NM_003430, AC006033, AF111848, AK025272, AL137077, L24498, NM_003590, NM_005774, and NM_014111.

43. The method of Claim 25, wherein said tobacco product is a cigarette.

44. A tobacco product made by the method of Claim 25.

45. A method of reducing the potential of a tobacco consumer to acquire a tobacco-related disease comprising providing the tobacco product made by the method of Claim 25 to said tobacco consumer.

46. Use of the method of Claim 25 to prepare a tobacco product that has a reduced potential to contribute to a tobacco-related disease.

47. A method to identify a gene that is modulated by exposure to tobacco smoke or a tobacco smoke condensate comprising:

providing a first isolated population of human bronchial epithelial cells (NHBE cells);

contacting said NHBE cells with tobacco smoke or a tobacco smoke condensate; and

identifying a gene that is modulated in response to contact with said tobacco smoke or said tobacco smoke condensate.

48. The method of Claim 47, wherein the expression of said gene is up-regulated.

49. The method of Claim 47, wherein the expression of said gene is down-regulated.

50. The method of Claim 47, wherein an oligonucleotide array is used to identify said gene that is modulated after said NHBE cells are contacted with said tobacco smoke or said tobacco smoke condensate.

51. The method of Claim 47, further comprising:

providing a second population of isolated human bronchial epithelial cells (NHBE cells), which are not contacted with an amount of tobacco smoke or tobacco smoke condensate; and

comparing the level of expression of at least one gene of said second population of NHBE cells with the level of expression of the same gene in said first population of NHBE cells, which has been contacted with said tobacco smoke or said tobacco smoke condensate so as to identify the modulation of a gene of said first population of NHBE cells that has been contacted with said tobacco smoke or tobacco smoke condensate.

52. A method to identify a predilection to acquire a tobacco-related disease in a subject comprising:

identifying a subject in need of a determination of a predilection to acquire a tobacco-related disease;

obtaining a biological sample from said subject; and

measuring the level of expression in said biological sample of at least one

gene selected from the group consisting of: FRHUL ferritin light chain, Ferritin, heavy polypeptide 1, Glutamate-cysteine ligase, catalytic subunit, Glutamate-cysteine ligase modifier subunit, Glutaredoxin (thioltransferase), Glutathione peroxidase 2, Glutathione reductase, Heme oxygenase 1, Jun D proto-oncogene, Microsomal glutathione S-transferase 3, NAD(P)H dehydrogenase, quinone 1, N-myc downstream regulated gene 1, Nuclear factor (erythroid-derived 2)-like 2, PDZ and LIM domain 1, Peroxiredoxin 1, S-adenosylhomocysteine hydrolase, Thioredoxin, Thioredoxin reductase 1, V-maf musculoaponeurotic fibrosarcoma oncogene homolog F, V-maf musculoaponeurotic fibrosarcoma oncogene homolog G, Amphiregulin (schwannoma-derived growth factor), Apoptosis related protein APR-3, Aurora-A kinase interacting protein, BH3 interacting domain death agonist, Calpain 1, (mu/I) large subunit, CDK4-binding protein p34SEI1, Cell death-regulatory protein GRIM19, Cysteine-rich, angiogenic inducer 61, DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5, Dickkopf homolog 3, Dual specificity phosphatase 5, Dual specificity phosphatase 6, EphA2, FAT tumor suppressor homolog 1, Granulin, Growth arrest and DNA-damage-inducible alpha, Histone deacetylase 3, Immediate early response 3, Immediate early response 5, Interleukin 1-beta, Kruppel-like factor 5, Myeloid/lymphoid or mixed-lineage leukemia trithorax homolog, Placental growth factor, vascular endothelial growth factor-related protein, Plasminogen activator, urokinase, PR domain containing ZNF domain, PRKC, WT1 regulator, Protein phosphatase 1, regulatory subunit 15A, Putative lymphocyte G0/G1 switch gene, Rho GTPase activating protein 4, Serum-inducible kinase, SKB1 homolog, Suppressor of G2 allele of SKP1, homolog of Testis enhanced gene transcript (BAX inhibitor 1), V-myc myelocytomatosis viral oncogene homolog, disintegrin and metalloproteinase domain 8, BCL2-associated athanogene 3, Chaperonin containing TCP1, subunit 5 (epsilon), Chaperonin containing TCP1, subunit 7 (eta), GABA(A) receptor-associated protein, DnaJ (Hsp40) homolog, subfamily A (member 1), DnaJ (Hsp40) homolog, subfamily B (member 1), DNAJ, Heat shock 105kD, Heat shock 10kD protein 1 (chaperonin 10), Heat shock 70kD protein 1A, Heat shock 70kD protein 8, Heat shock 70kD protein 9B (mortalin-2), Heat shock protein 75, Stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizing protein), Matrix metalloproteinase 14 (membrane-inserted), Proteasome (prosome,

macropain) 26S subunit, ATPase, 1, Proteasome (prosome, macropain) 26S subunit, ATPase 6, Proteasome (prosome, macropain) 26S subunit, non-ATPase, 11, Proteasome (prosome, macropain) 26S subunit, non-ATPase 8, Proteasome (prosome, macropain) subunit, beta type 5, Proteasome (prosome, macropain) subunit beta type 7, Protein translocation complex beta, Ring-box 1, Sequestosome 1, Signal recognition particle 14kD (homologous Alu RNA binding protein), Tetratricopeptide repeat domain 1, Thyroid hormone receptor interactor 12, Ubiquitin C, Ubiquitin carrier protein, Ubiquitin-activating enzyme E1 (A1S9T and BN75 temperature sensitivity complementing), Ubiquitin-conjugating enzyme E2 variant 1, Ubiquitin-conjugating enzyme E2M (UBC12 homolog), 3-hydroxy-3-methylglutaryl-Coenzyme A reductase, 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble), Acetyl-Coenzyme A acetyltransferase 2 (acetoacetyl Coenzyme A thiolase), Annexin A1, Cytosolic acyl coenzyme A thioester hydrolase, Diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein), Enoyl Coenzyme A hydratase, short chain, 1, mitochondrial, Fatty acid synthase, Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase, Insulin induced gene 1, Isopentenyl-diphosphate delta isomerase, Niemann-Pick disease, type C1, Phosphatidic acid phosphatase type 2B, Prosaposin (variant Gaucher disease and variant metachromatic leukodystrophy), Sterol regulatory element binding transcription factor 2, Sterol-C4-methyl oxidase-like, and Sterol-C5-desaturase (ERG3 delta-5-desaturase homolog)-like, whereby a level of expression of said at least one gene that differs from the level of expression of the same gene in a biological sample obtained from a second subject that has not consumed tobacco indicates that said subject has a predilection to acquire a tobacco-related disease.